Evol Ecol (2010) 24:1017–1030
DOI 10.1007/s10682-010-9368-3

ORIGINAL PAPER

Insect oviposition behavior affects the evolution of adaptation to *Bt* crops: consequences for refuge policies

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Received: 8 October 2009 / Accepted: 3 February 2010 / Published online: 18 February 2010

Abstract The major lepidopteran insect pests of cotton and maize harbor intra-specific variation for behavior determining the selection of host plants for oviposition. Yet, the consequences of behavioral adaptation for fitness have neither been modeled nor monitored for *Bt* cotton and maize crops, the most widely grown transgenic herbivore-resistant plants. Here, we present a general two-locus heuristic model to examine potential outcomes of natural selection when pest populations initially have low frequencies of alleles for both physiological and behavioral adaptation to *Bt* crops. We demonstrate that certain ecological conditions allow for the evolution of behavioral choices favoring alternative oviposition hosts that limit the increase in resistance alleles, even when they are phenotypically dominant. These results have implications for current refuge policies, which should be adapted to promote the evolution of certain behavioral choices for alternative oviposition hosts in addition to dilution of physiological resistance alleles. Collection of data on oviposition host preference as a component of monitoring schemes will provide important insights into mechanisms underlying the durability of *Bt*-transgenic host-plant resistance.

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Keywords Behavioral resistance · Physiological resistance · *Bt* crop · Monitoring · Host specificity · Host shift

Introduction

Over the last two decades, transgenic corn and cotton crops that are resistant to insects as a result of the expression of *Bacillus thuringiensis* (*Bt*) toxin genes have been introduced worldwide. In some areas in the US, China, India and South Africa they have virtually replaced non-*Bt* crop cultivars, especially in the case of cotton (Carrière et al. 2005; James 2008; Wu 2007; Wu et al. 2008; Jaffe 2009; Kruger et al. 2009). This has created a situation in which a host plant species, that is suitable for pest growth (when not treated with insecticide), has suddenly for some pests changed into a trap crop with virtually no surviving offspring except in those cases where deliberately planted, non-*Bt* cultivars occupy 4–20% of the crop area and create refuges for the insects. In some countries, for *Bt* corn and cotton cultivars that produce a single *Bt*-toxin, the deliberate planting of non-*Bt* cultivars of the same crop species is enforced by law (Carrière et al. 2005; Jaffe 2009; Kruger et al. 2009). These refuges were designed based on general population genetic models which predicted that such refuges would substantially decrease the rate at which pest populations evolved physiological resistance to the toxins (Kennedy et al. 1987; Tabashnik 1994; Gould 1998). Since then, more complex models that include spatial insect population structure and multiple plant species ecosystems have supported the predicted utility of refuges, especially for transgenic crops expressing *Bt* toxins at high levels (Peck et al. 1999; Storer et al. 2003; Sisterson et al. 2005; Shelton et al. 2008).

Recently, a debate arose regarding the empirical evidence of field-evolved insect resistance to *Bt*-crops (Moar et al. 2008; Tabashnik et al. 2008a, b). The authors discussed the results of more than a decade of global monitoring of the occurrence of insect resistance to *Bt*-crops for different insect species. They established regional differences, but overall, the development of resistance (or even higher tolerance) to *Bt* crops has been limited if worrying (Tabashnik et al. 2009). The following explanations have been presented for the low incidence of resistance: (1) insecticides have been applied to the *Bt* crops, which may render *Bt* crops with moderate expression of the *Bt* toxin more like a high dose cultivar (Jackson et al. 2004); (2) incomplete resistance of insects to *Bt* toxins is often observed in the laboratory strains of pests and creates a fitness cost as even insects that are 100-fold less sensitive may still suffer 50–60% mortality on *Bt*-plants with high toxin levels (Tabashnik et al. 2008b); (3) gene pyramiding (combining several *Bt*-genes in one plant) has been practiced since 2002, further reducing the likelihood of resistance development for some insect species; (4) lower than estimated initial resistance allele (R-allele) frequencies may prolong the development of resistance; (5) recessive inheritance of resistance to *Bt* crops occurs more frequently than dominant inheritance; and (6) refuges additional to non-*Bt* host plants are often available (similar or different crops and weeds) (Tabashnik et al. 2003, 2005, 2008b). It is argued that the experimental data are consistent with the theory underlying the refuge strategy, because the exceptional cases of resistance to *Bt* toxins have occurred when the concentration of *Bt* toxin in the crops cause intermediate mortality of the pest, and the refuge strategy does not protect against resistance for long periods of time in such cases (Burd et al. 2000; Tabashnik et al. 2009).

In all of these recent discussions there is no mention of insect behavior as a factor to consider. Yet, there is extensive experimental evidence for considerable genetic variation

in oviposition preference among major lepidopteran cotton and maize pests (Schneider and Rousch 1986; Fitt 1991; Jallow and Zalucki 1996; Jallow et al. 2004; Wang et al. 2004; Malausa et al. 2008). Although natural selection for behavioral selection of plants for oviposition as a mechanism of herbivore adaptation to classical host-plant resistance has been analyzed with quantitative models (Gould 1984; Kennedy et al. 1987; Castillo-Chavez et al. 1988), and was briefly mentioned with regard to *Bt* crops (Gould 1988; Jallow et al. 2004; Tabashnik et al. 2009), the potential impacts of behavioral adaptation in cropping systems that include *Bt* crops have received little attention.

Oviposition behavior should be considered in refuge models

The suitability of crop hosts for pest species is often compromised when crop economics and general farming practices result in heavy use of insecticides, but the recent commercialization of *Bt* cotton and corn effectively decrease survival of some pest species to zero, turning a host plant into a trap crop or sink (Carrière et al. 2003; Wu et al. 2008). Little attention has been given in the literature to the fact that the huge areas planted with *Bt*-corn and -cotton exert strong selection in favor of moths with a genetically controlled preference for non-*Bt* alternative crops and/or weeds. The evolution of behavioral preference for cultivars without *Bt* toxin genes over cultivars carrying *Bt* toxin genes is not expected due to the inability of female moths to sense the expression of the *Bt* toxin protein in plant cells and the absence of any other consistent phenotypic differences between *Bt* and non-*Bt* cultivars (Tate et al. 2006).

If, within a population, the only insects that survive are those that feed on alternative crops or weeds, selection pressure favoring behavioral avoidance of the *Bt* crop species or strong preference for one or more of the suitable alternative host plants is expected to be strong. If strong behavioral preference for alternative oviposition hosts evolved before physiological resistance, then selection for resistance to the *Bt* crops could be very low and, as a consequence, the proportion of insects carrying *R*-alleles could remain at background levels. Furthermore, any increase in R-allele frequency, e.g. due to a temporarily insufficient availability of refuges, will be transient, because allele C, determining the choice of oviposition hosts, will direct the offspring in large majority to alternative crops and weeds when they recur (Badenes-Perez et al. 2004; Feder and Forbes 2007). The recent introduction of cultivars with multiple *Bt* toxins that differ in mode of action is expected to make physiological adaptation less likely (Gould et al. 2006), and, therefore, increases the potential for behavioral adaptation.

Some insect pest species such as the pink bollworm (*Pectinophora gossypiella*) and the rice stem borers *Chilo suppressalis* and *Scirpophaga incertulas* that are current or proposed targets of *Bt* crop cultivars are highly specialized in their host use, and therefore have few alternative hosts. In these cases, behavioral selection of alternative oviposition hosts would be a less likely evolutionary scenario. However, other pests of *Bt* crops such as the cotton bollworms (*Helicoverpa zea*, *H. armigera and H. punctigera*) and the tobacco budworm (*Heliothis virescens*) have broad host ranges that include many plant families, some of which are other crops or weeds within cotton or maize cropping systems. Because these alternative crops and weeds are already used for oviposition by female moths, a genetic change that increases the preference for these host plants is to be expected.

Here, we propose that evolutionary change in insect behavior determining the selection of plants for oviposition based on one or more rare preference and/or avoidance alleles (for the sake of simplicity grouped together as oviposition choice allele C) could be as important as physiological resistance in some pest/crop systems. The potential implications for monitoring and refuge policies are discussed.

Materials and methods

Two-locus population genetics model

This model tracks the frequencies of all 9 possible genotypes. Let f_{ij} be the frequency of genotype *(i, j)* at generation t, where $i = RR$, Rr or rr, and $j = CC$, Cc or cc. The R allele codes for physiological resistance and the C allele codes for oviposition choice of alternative hosts. The frequencies of the four possible gametic types are given by:

$$g_{RC} = f_{RRCC} + \frac{1}{2}f_{RRCc} + \frac{1}{2}f_{RrCC} + \frac{1}{4}f_{RrCc}$$
$$g_{Rc} = \frac{1}{2}f_{RRCc} + f_{RRcc} + \frac{1}{4}f_{RrCc} + \frac{1}{2}f_{Rrcc}$$
$$g_{rC} = \frac{1}{2}f_{RrCC} + \frac{1}{4}f_{RrCc} + f_{rrCC} + \frac{1}{2}f_{rrCc}$$
$$g_{rc} = \frac{1}{4}f_{RrCc} + \frac{1}{2}f_{Rrcc} + \frac{1}{2}f_{rrCc} + f_{rrcc}$$

We first calculate the genotypic frequencies at generation $t + 1$ before selection (noted f_{ij}'). This model assumed an infinite, totally panmictic, population with no age or spatial structure. The frequencies correspond therefore to Hardy–Weinberg equilibrium for the two unlinked loci, and are given by:

$$f'_{RRCC} = g_{RC}^2$$
$$f'_{RRCc} = 2g_{RC}g_{Rc}$$
$$f'_{RRcc} = g_{Rc}^2$$
$$f'_{RrCC} = 2g_{RC}g_{rC}$$
$$f'_{RrCc} = 2g_{RC}g_{rc} + 2g_{Rc}g_{rC}$$
$$f'_{Rrcc} = 2g_{Rc}g_{rc}$$
$$f'_{rrCC} = g_{rC}^2$$
$$f'_{rrCc} = 2g_{rC}g_{rc}$$
$$f'_{rrcc} = g_{rc}^2$$

These frequencies are then adjusted based on the relative fitness of each genotype in the environment being considered (see below). The relative fitness of genotype (i, j) is calculated as the sum of the fitnesses of this genotype on all available crops, weighed by the proportion of oviposition occuring on each type of crop:

$$w_{ij} = \sum_{k \text{ crop types}} (\% \text{ oviposition on crop } k)\,(\text{fitness } (i,j) \text{ on crop } k)$$

Here, there are three possible crop types: *Bt* cultivar of the target crop, non-*Bt* cultivar of the target crop, and alternative crop or weed. Table 1 defines the fitness values and oviposition proportions for different scenarios or environments being considered.

The total fitness of the population is given by:

$$\overline{w} = \sum_{(i,j)} w_{ij} f_{ij}$$

and, for each genotype *(i, j)*, the frequency at generation $t + 1$ after selection, noted f_{ij}'', is given by:

Table 1 Parameters used to model evolution of resistance to *Bt* toxins in transgenic crops by a generic insect pest under the influence of a single locus R for physiological resistance to *Bt* toxins or a double locus R/C with additional behavioral choice C of an alternative oviposition host under both recessive or dominant inheritance

Model para meters	Single locus R[a]			Double locus R & C[a]											
	Generic insect			Generic insect with C				Generic insect with C and high fitness on *Bt* crops[b, c]							
	S1	S2	S3	S4	S5	S6	S7	S8rd/dd	S9rd/dd	S10rr	S10rd	S10dd	S11rr	S11rd	S11dd
Bt crop fitness[d]															
BtW_{RR}	04	0.4	0.4	0.4	0.4	0.4	0.4	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
BtW_{Rr}	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.8	0–0.8	0	0	0.8	0.2	0.2	0.8
BtW_{rr}	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2	0.2
Alternate refuge fitness[d]															
$altRefW_{RR}$	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.4	0.4	0.4	0.4	0.4	0.4
$altRefW_{Rr}$	1–0.8	1–0.8	1–0.8	1–0.8	1–0.8	1–0.8	1–0.8	1–0.8	1–0.8	0.5	0.5	0.4	0.5	0.5	0.4
$altRefW_{rr}$	1	1	1	1	1	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
Isogenic refuge fitness[d]															
$isoRefW_{RR}$		0.8	0.8				0.8		0.8				0.8	0.8	0.8
$isoRefW_{Rr}$		1–0.8	1–0.8				1–0.8		1–0.8				1	1	1
$isoRefW_{rr}$		1	1				1		1				1	1	1
Percentage on *Bt* crop															
$ChsAlt_{cc}$	99	95	80	99	99	99	80	99	80	99	99	99	80	80	80
$ChsAlt_{Cc}$	–	–	–	20	5	1	1	1	1	99	1	1	80	1	1
$ChsAlt_{CC}$	–	–	–	20	5	1	1	1	1	1	1	1	1	1	1
Percentage refuge[e]															
Isogenic crop	0	4	19	0	0	0	19	0	19	0	0	0	19	19	19
Alternate crop	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Initial freq R	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Initial freq C	0	0	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 1 continued

Model para meters	Single locus R[a]			Double locus R & C[a]											
	Generic insect			Generic insect with C				Generic insect with C and high fitness on *Bt* crops[b, c]							
	S1	S2	S3	S4	S5	S6	S7	S8rd/dd	S9rd/dd	S10rr	S10rd	S10dd	S11rr	S11rd	S11dd
Model results after 1,000 generations															
Final freq recessive R	1	1	1	0.001	0.001	0.001	0.001	0.001	0.001	1	0	–	1	0.001	–
Final freq dominant R	0.999	0.999	0.889	0	0	0	0	0	0	–	–	0.999	–	–	0.999
Final freq recessive C	–	–	–	–	–	–	–	–	–	0.001		–	0.001	–	–
Final freq dominant C	0	0	0	0.999	0.999	0.999	0.999	0.999	0.999	–	0.999	0	–	0.998	0

[a] Generic insect parameters were taken from Tabashnik et al. (2008a, b). The double values (0–0.4; 1–0.8) with some of the heterozygous genotypes BtW_{Rr}, $altRefW_{Rr}$, $isoRefW_{Rr}$ indicate a fitness of 0 (*Bt*) or 1 (alt/iso) if R is recessive and a fitness of 0.4 (*Bt*) or 0.8 (alt/iso) if R is fully dominant

[b] S8–S11 have suffixes which represent combinations of either recessive (r) or dominant (d) physiological resistance R, and recessive (r) or dominant (d) behavioral choice C in combinations of rr, dd and rd of RC

[c] Only S10rr and S11rr employ a recessive notation of behavioral avoidance

[d] W is the symbol for fitness

[e] Percentage refuge represents the percentage of oviposition on the refuge crop or weed if the C allele is not present

$$f''_{ij} = \frac{w_{ij} f'_{ij}}{\overline{w}}$$

Scenario parameters

The model was run for a series of 11 scenarios with variation in different parameters. The scenarios themselves are discussed in the Results section. Here, the general meaning and choice of the parameters as listed in Table 1 are described. "*Bt* crop fitness" represents the insect fitness on the *Bt* crop and is given as BtW_{RR}, BtW_{Rr}, and BtW_{rr} for the different *Bt* crop-relevant genotypes. The chosen fitness parameters are based on those used for the generic lepidopteran insect by Tabashnik et al. (2008b). The heterozygote BtW_{Rr} possesses a fitness of 0 if the resistance to *Bt* toxin is recessive and 0.4 if it is dominant in the different runs of the model. These values are raised in the scenarios S8–S11. The "Alternate refuge fitness" parameters $altRefW_{RR}$, $altRefW_{Rr}$, and $altRefW_{rr}$, represent the fitness of a generic lepidopteran insect on a completely different crop species or a weed, relative to its fitness on the *Bt* crop. Tabashnik et al. (2008b) do not take such crops into account, but our simplest assumption is that the fitness is the same as the "Isogenic refuge fitness" $isoRefW_{RR}$, $isoRefW_{Rr}$, $isoRefW_{rr}$, (the same crop, but without *Bt*) for which Tabashnik et al. (2008b) did provide data: 0.8 for the homozygous resistant genotype RR and 1 for the homozygous sensitive genotype rr. In scenarios S10–S11 we reduce those fitness values to test the effects this has on our model predictions. The reduced fitness of the homozygous resistant genotype RR relative to the sensitive genotype rr is due to the fact that sometimes the resistance gene carries a cost which can occur already in the heterozygote genotype Rr if it is dominant (0.8) but which is absent when it is recessive (1.0). "Percentage Refuge" in Table 1 shows that isogenic refuges are not incorporated in models S1, S4–S6, S8, and S10, and here the model assumes that, if the moth population does not possess the choice allele C to choose alternate crops over *Bt*- and isogenic crops, i.e. when all moths are genotype $ChsAlt_{cc}$,as in S1–S3, that 99% of all moths will oviposit on the *Bt*- or isogenic crop and only 1% on the available alternate crop. The presence of an isogenic crop refuge of 4% (in S2) or 19% (in S3) will reduce the percentage of adults ovipositing on the *Bt* crop from 99 to 95 and 80% respectively as in "Percentage on *Bt* crop" on the basis of the planting regime and not due to choice. The introduction of a dominant oviposition choice allele C in genotypes $ChsAlt_{Cc}$ and $ChsAlt_{CC}$ confers those genotypes the ability to choose alternate crops to the extent that only 20, 5 or 1% (scenarios S4–S6) will still oviposit on the *Bt* crop. Allele C is a Genotype × Environment term in our model as it does not separately take into account host plant density and distribution as a factor contributing to the choice of oviposition hosts. The initial frequency of the R gene ("Initial freq R") was chosen to be 0.001 as in Tabashnik et al. (2008b). The initial frequency of C ("Initial freq C") was arbitrarily chosen to be 0.001 as well, as only little is known about the real frequencies of oviposition preference genes. With both genes assumed to be equally rare the effect of frequency is eliminated as a dominant factor contributing to the observed outcomes of the model. Some specific situations occur in scenarios S10rr and S11rr where the C gene is assumed to be recessive. The $ChsAlt_{Cc}$ genotypes are unable to choose the alternate crop for oviposition and as a result still oviposit 99% (S10rr) or 80% (S11rr) on the *Bt* crop, depending the presence of an isogenic refuge. Finally, Table 1 provides the population frequencies, which the model returned after 1,000 generations, for both recessive and dominant R and C genes.

Results

Two locus genetic models predict suppression of both recessive and dominant physiological resistance alleles

To illustrate the potential effect of an oviposition host choice allele C on the suppression of a resistance allele we ran a two-locus model to examine the parameter space governing suppression of recessive or dominant *R* alleles, and to study the implications for the current refuge policy.

The population-genetics model used for the simulations was similar to the model used by (Gould 1984; Castillo-Chavez et al. 1988). The model assumes two unlinked loci: R for physiological resistance, and C for behavioral oviposition choice, with each locus having two alleles. The model does not include population dynamics or population structure, so it assumes random mating and produces Hardy–Weinberg proportions of genotypes when there is no selection. The model allows insertion of a range of estimates of relative genotype fitnesses. The genotype with the highest fitness in any simulation has its fitness set at 1.0. Some population genetic models of behavior-mediated host-range evolution have assumed soft selection; density dependence on each host (Wallace 1968; Rausher 1985), while others have assumed hard selection; no density dependence or only density dependence of the entire insect population (Castillo-Chavez et al. 1988). Soft selection can be defined as a situation where there is strong density dependent competition or predation that maintains an upper limit on the population density within a specified habitat (e.g., insects on one of the host plants). For some pest species intraspecific competition and/or density-dependent predation on alternative hosts could result in a system with soft selection (Storer et al. 2003). Here, we chose to use a model with hard selection, because data needed to predict the shape of density dependent relationships as pest populations shift to alternative host plants are not available. We do recognize that the assumption of hard selection results in more extreme outcomes (Gould 1984; Rausher 1985).

The starting point for the choice of genotype-specific fitness parameters was based on the generic insect example used by (Tabashnik et al. 2008b). Table 1 provides an overview of the model parameters used for each of 11 simulated scenarios. Figures 1, 2, and 3 present graphical illustrations of the time course of R- and C-gene frequencies over 1,000 generations for both the recessive and dominant R/C allele scenarios. Scenarios S1–S3 show predictions based on the presence of an isogenic non-*Bt* crop refuge on the current single locus models for recessive and dominant *R* alleles. These models assume that the refuge cannot be discriminated reliably by the female moth and indeed current refuge policies assume just this sort of scenario. Scenarios S4–S7 show the effects of a rare behavioral oviposition host choice allele C, which allows female moths to discriminate in favor of alternative non-*Bt* crops or weeds to varying degrees, ranging from 80 to 99%. Scenarios S8–S11 investigate the effects of increasing the fitness of the generic insect on *Bt* hosts, lowering the fitness on the alternative hosts, when the C-allele is either dominant or recessive.

Scenarios S1–S3 based on insect populations with only potential for physiological adaptation

Scenario S1 represents the most extreme situation of a 100% *Bt* crop with no refuge other than alternative crops or weeds. Due to our model assumption of large monocultures of cotton or corn this results in 99% of the insect population ovipositing on the *Bt* crop and only 1% on the available alternative crop or weed. Under these conditions, when the R

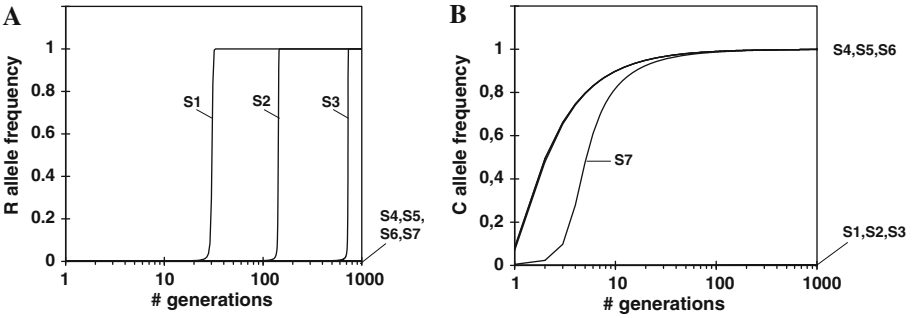

Fig. 1 The effects of alternative oviposition host preference behavior on *recessive* R and dominant C allele frequencies. S1–S3 represent scenarios without, and S4–S7 scenarios with alternative oviposition host choice of *Bt* crops by a dominant C allele (see Table 1 and M&M for the full parameter overview). Scenarios S4–S7 result in stable polymorphisms with 80–99% of the insect populations on alternative hosts, and with full suppression of R at all times. The *panel A* shows the R allele and the *panel B* the C allele frequencies as a function of generation number. All scenarios assume initial frequencies of 0.001 of both R and C

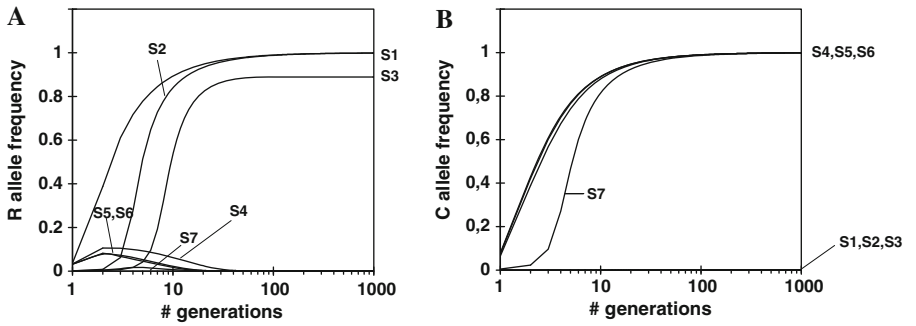

Fig. 2 The effects of alternative oviposition host preference behavior on *dominant* R and C allele frequencies. S1–S3 represent scenarios without, and S4–S7 scenarios with alternative oviposition host choice by a dominant C allele (see Table 1 and M&M for the full parameter overview). Scenarios S4–S7 result in stable polymorphisms with 80–99% of the insect populations on alternative hosts, and with full suppression of R after an initial rise. The *panel A* shows the R allele and the *panel B* the C allele frequencies as a function of generation number. All scenarios assume initial frequencies of 0.001 of both R and C

allele is recessive it takes 31 generations for the allele frequency in the population to reach 0.50, and only 3 generations when the R allele is dominant. Both recessive and dominant *R* alleles are, therefore, predicted to reach fixed frequencies rapidly in the population in this single-locus model.

Scenarios S2–S3 represent the current policy-enforced situation in the US of a single-gene *Bt* crop cultivar grown together with a 4–20% non-*Bt* refuge of a related cultivar. A fixed proportion of the insect population is assumed to always oviposit on the *Bt* crop as moths cannot discriminate between the toxic and non-toxic crop, thus maintaining selection pressures. It takes 142 generations in S2 (4% refuge) and 725 generations in S3 (20% refuge) to resistance in a recessive single-locus model (Fig. 1) which is a 4- to 23-fold delay compared to scenario S1. However, dominant *R* alleles are virtually insensitive to this kind of refuge and already after 4–9 generations (1–3 fold delay) dominant *R* alleles will emerge from background levels despite the refuge (Fig. 2).

Scenarios S4–S7 based on insect populations with the alternative oviposition host choice locus C

Scenarios S4–S6 show the effect of assuming a dominantly inherited, rare behavioral alternative oviposition host choice allele C in the model. Such genetic variation for host selection has been described for the generalist herbivores of cotton and maize such as *Helicoverpa*, *Heliothis* and *Ostrinia* species (Schneider and Rousch 1986; Jallow et al. 2004; Malausa et al. 2008). Even a very small subset of 20 randomly selected *H. armigera* females, that were given a choice of 6 different plant hosts in a glasshouse, resulted in three quarters of the females ovipositing as much as 38% of their eggs on maize plants compared to another quarter exhibiting much lower maize preferences for maize (as few as 1.8% of eggs on maize) (Jallow and Zalucki 1995). The F1 offspring inherited this preference (Jallow and Zalucki 1996). This 20-fold difference, classified here as a percentage of 5% on the *Bt* crop for Cc and CC genotypes relative to normally 99% for cc genotypes, is already found among 20 individuals and is found using an experimental set-up which mostly determines the post-alighting host selection based on gustatory and mechanical cues. If one includes the effects of pre-alighting olfactory and visual cues operational in open fields, and would draw from much larger populations (1,000 individuals as practised in the model described here) higher selectiveness of e.g. 99% for different ovipositon hosts possibly exist in individual cases.

Remarkably, Scenario 4 shows that already if 1 in 1,000 insects carries an allele C which directs on average 80% of the females to avoid the *Bt* crop and/or prefer to oviposit on the alternative crop or weed, this completely reverses the predictions of the recessive (Fig. 1) and dominant (Fig. 2) R-allele scenarios of S1. Despite the absence of any instituted refuge, recessive physiological resistance alleles are completely suppressed, and dominant alleles are stabilized at a level controlled by the degree of alternative oviposition host choice caused by the C allele. An alternative oviposition host choice of 80% (=in Table 1, percentage on *Bt* crop: 20% by ChsAltCc and ChsAltCC genotypes) in this case results in a stable R-allele frequency of 0.1%, due to the fact that nearly 100% of the population has acquired the dominant C allele in only 3 generations (Fig. 2b), and, consequently, the majority (80%) of insects carrying this allele are no longer exposed to *Bt*-toxins. If a more effective alternative oviposition host choice allele exists in the population resulting in 95% of adults selecting the non-*Bt* alternative crop or weed (=5% on *Bt* crop), as in scenario S5, or 99% (=1% on *Bt* crop) as in scenario S6 the suppression of the dominant R-allele, as expected, also remains at the same background levels. Thus, in all settings both recessive and dominant resistance alleles will always remain at background levels of 0.1%. As a result, a pest population is developing which no longer oviposits on *Bt* crops. Of course this also reduces the population size to levels which can be sustained on the available alternative host plants and this could change the average fitness of individuals on the alternative hosts due to density dependent effects. Scenario S6 represents an extreme scenario dependent on 100% implementation of any specific *Bt* crop. However, despite the fact that during the past 13 years in the US *Bt* crops represented a growing proportion of the planted area, on a larger scale and under current policies they have stayed well below 100% especially in corn (Carrière et al. 2005; James 2008; Jaffe 2009). It is, therefore, relevant to model the effects of a large non-*Bt* refuge of the cotton or corn crop as in scenario S3, but with the assumptions of scenario S6. As can be seen in Fig. 2, scenario S7 does not yield a significantly different result compared to S6 and initially suppresses dominant R-allele emergence even better. Both scenarios rapidly result in background R-allele frequencies of 0.1 when 99% of pest insects choose the crop refuge.

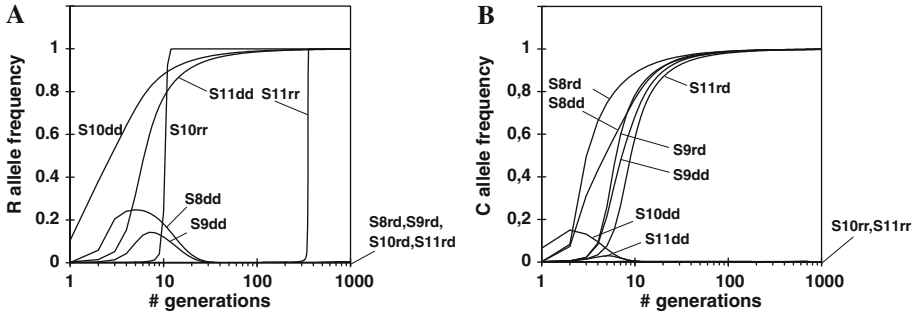

Fig. 3 The effects of alternative oviposition host preference behavior under more stringent conditions, i.e. double fitness of generic insects on *Bt* crops (S8) and halved fitness on the alternative crop or weed (S9), also varying the dominance and recessiveness of the alternative oviposition host choice trait C (S10–11). Shown are combinations of either recessive (r) or dominant (d) physiological resistance R and recessive (r) or dominant (d) alternative oviposition host choice allele C in combinations of rr, dd and rd of RC. Alternative refuge fitness is half that of isogenic refuge fitness in scenarios S10–S11. The *panel A* shows the R allele and the *panel B* the C allele frequencies as a function of generation number. All scenarios assume initial frequencies of 0.001 of both R and C

Scenarios S8–S11 based on physiologically resistant insects having higher fitness on *Bt* crops than on alternative hosts, and a recessive C allele

In order to test the robustness of the two-locus model for suppressing the emergence of the R allele we ran the model with combinations of (1) doubled fitness of the insects on the *Bt* crop, (2) in addition to (1) halved fitness on the alternative crop, and/or (3) recessiveness of the C allele. Wildtype fitness on *Bt* crops of RR genotypes has not been observed with *Helicoverpa armigera, Helicoverpa zea,* and *Pectinophora gossypiella* ($BtW_{RR} = 0.161$–0.404), but is commonly seen with *Heliothis virescens, Sesamia nonagrioides* and *Ostrinia nubilalis* ($BtW_{RR} = 1$) (Tabashnik et al. 2008b). To accommodate for those species, scenarios with doubled fitness (S8/S9) were modeled. Fitnesses of the RR genotypes on near isogenic non-*Bt* crops are generally close to 1 for most of these pests (iso-$RefW_{RR} = 0.46$–1.0) (Tabashnik et al. 2008b), but could be much lower on alternative crops or weeds ($altRefW_{RR} = 0.4$). Those scenarios were modeled in S10/S11. Finally, a recessive C allele could be one which represents a loss instead of an evolved ability to discriminate a positive pre- or post-alighting cue associated with a host plant. Those scenarios were modeled in S10rr and S11rr where the suffixes rr denote the recessiveness of both R and C (in other scenarios rd and dd denote recessive R dominant C, and dominant R and C, respectively).

Scenario S8 in Fig. 3 only differs from scenario S6 in terms of double fitness relative to the generic insect on the *Bt* crop. Also under that scenario, recessive *R* alleles remain at background levels (Fig. 3a, S8rd), but R allele frequencies are more responsive to dominant alleles in insects with higher fitness. Initially, during the first 10 generations of scenario S8dd they become manifest (Fig. 3a) up to a frequency of 25%. However, the even more rapid emergence of the dominant C allele (Fig. 3b) can finally fully offset the rise of the R allele. Scenarios S8rd and S8dd more closely resemble the parameters described for *H. virescens, S. nonagrioides* and *O. nubilalis* (Tabashnik et al. 2008b), suggesting that for those realistic cases a non-*Bt* cultivar refuge requirement would not be mandatory to keep either recessive or dominant *R* alleles at low frequencies.

In S9 we tested the effect of the currently applied refuge on S8rd and S8dd. Figure 3a shows that S9rd is still fully repressing the recessive R allele, and that the S9dd scenario is even more effective than S8dd without refuge in repressing the dominant R allele. Thus, the currently enforced refuge plantings appear to have done no harm, although the model predicts such plantings could have been removed after the first 10–20 generations, when the C allele has achieved dominance in the population.

If on top of the double fitness on the *Bt* crops, the fitness on the alternative crop is halved and the alternative oviposition host choice locus is made recessive, the recessive allele C can no longer control the recessive R allele as shown in scenario S10rr. Similarly, a dominant C allele in S10dd is not effective in controlling a dominant R allele, although it still fully controls the most commonly observed recessive R allele in S10rd. If such extreme situations apply to particular agricultural areas, our model predicts that planting of specific alternative crops with better fitness prospects (S8) could offer a more durable solution for controlling R gene emergence than planting the near isogenic crop as practiced now, as that approach suffers from maintaining high selection pressures on insects colonizing the *Bt* crop.

In scenario S11 the fitness of the generic insect on *Bt* crops is assumed to be further improved with survival of rr insects ($BtW_{rr} = 0.2$) even without the R allele compared to S10. This situation applies to *H. armigera* and *H. zea* in the field, although with lower recorded values of 0.047 and 0.1 respectively for the rr, and much lower fitnesses for the rR and RR genotypes according to (Tabashnik et al. 2008b). Compared to S10 the effect of higher fitness combined with a refuge of 20% is modeled, and it is clear that under those two conditions the appearance of the R allele can be delayed in both the double recessive (351 compared to 10 generations) and double dominant (7 compared to 4 generations) case. If then, in scenario S11rd, the usually observed recessive R allele is combined with the dominant C allele a stable polymorphism results with full suppression of the R allele at background levels.

Thus, the alternative oviposition host choice allele C not only represses the physiological resistance allele R under the generic parameters modeled earlier by Tabashnik et al. (2008a, b), but also under much less favorable conditions which may apply to specific species of *Bt* resistant insects that are more fit on *Bt* crops, and less fit on alternative crops. Those cases, however, do depend on dominant *C* alleles under the chosen parameter regimes.

Discussion

This study shows that evolutionary changes in host-plant preference of insect pests should be considered as one factor that may account for the slower than expected physiological adaptation of these insects to *Bt* crops (Moar et al. 2008; Tabashnik et al. 2008a, b, 2009). The introduction of behavioral choice for alternative oviposition host-plants into the model shows that many of those predictions are potentially reversed by the generation of stable polymorphisms, which are unexpected based on single-locus models of physiological adaptation. In China where *Bt* cotton is planted on 3.8 million ha with a rapidly decreasing, non-mandatory cotton refuge; currently 0–20%, (Wu et al. 2008), the moths can take corn, peanuts, legumes and vegetables as alternative oviposition hosts. Cotton overall represents 10–14% of the total area of available host crops. Yet egg densities on cotton from second and third generation *H. armigera* moths reduced strongly (5–7 fold) over the period of 1997–2006 (Wu et al. 2008). On the alternative crops similar reductions were observed, but

remained unexplained. On the one hand, these reductions on both *Bt* cotton and alternative crops may reflect the increasing adoption rates of *Bt* cotton, resulting in increasingly reduced potential for reproduction on cotton. With cotton acting as a trap crop across subsequent generations in one season, these effects could amplify exponentially despite the relatively small proportion of potential hosts represented by cotton in the case of China. On the other hand, as shown by this paper, a changing host specificity, based on natural variation known to be present in this species, would be expected to start playing an increasingly important role in parallel (Jallow and Zalucki 1995, 1996). In the long run, it would predict increasing populations on the alternative crops, and further reduced oviposition rates on *Bt* cotton, when it loses its current function as a trap crop. Yet, the fact that *H. armigera* has to pass through several host shifts in subsequent generations each season could present evolutionary limits to behavioral change in this pest (1st generation on wheat, 2nd generation avoid *Bt* cotton, in favor of peanuts, legumes and vegetables—corn not yet available; 3rd and 4th generation avoid *Bt* cotton in favor of corn (largest acreage), legumes and vegetables). It is, furthermore, possible that the pests have reduced fitness on the alternative hosts, leading to a population, which is successful, but smaller on the alternative crops (Jallow et al. 2004). These factors could slow down the observed rate of behavioral change or could constrain the degree of host preference changes.

Unfortunately, *Bt* resistance monitoring programs across the world have not included the collection of relevant data on potential shifts in host preference of selected pests towards locally available alternative crops and weeds, so it is impossible to know whether there have been small to moderate shifts in host-plant preference that would in essence result in larger than predicted refuge size. Our modeling results are dependent on parameter choices for alternative hosts, some of which still need to be validated in the field. We strongly recommend that studies on shifts in host preference be undertaken in order to ensure that refuge policies are based on the most complete relevant biological information.

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